

## PERSPECTIVE

# Microbial contributions to the persistence of coral reefs

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On contemplating the adaptive capacity of reef organisms to a rapidly changing environment, the microbiome offers significant and greatly unrecognised potential. Microbial symbionts contribute to the physiology, development, immunity and behaviour of their hosts, and can respond very rapidly to changing environmental conditions, providing a powerful mechanism for acclimatisation and also possibly rapid evolution of coral reef holobionts. Environmentally acquired fluctuations in the microbiome can have significant functional consequences for the holobiont phenotype upon which selection can act. Environmentally induced changes in microbial abundance may be analogous to host gene duplication, symbiont switching / shuffling as a result of environmental change can either remove or introduce raw genetic material into the holobiont; and horizontal gene transfer can facilitate rapid evolution within microbial strains. Vertical transmission of symbionts is a key feature of many reef holobionts and this would enable environmentally acquired microbial traits to be faithfully passed to future generations, ultimately facilitating microbiome-mediated transgenerational acclimatisation (MMTA) and potentially even adaptation of reef species in a rapidly changing climate. In this commentary, we highlight the capacity and mechanisms for MMTA in reef species, propose a modified Price equation as a framework for assessing MMTA and recommend future areas of research to better understand how microorganisms contribute to the transgenerational acclimatisation of reef organisms, which is essential if we are to reliably predict the consequences of global change for reef ecosystems.

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## Coral reefs under climate change

Corals are fundamental ecosystem engineers, responsible for constructing large intricate reefs that support diverse and abundant marine life. Central to a healthy coral animal is a dynamic relationship with diverse eukaryotic, prokaryotic and viral symbionts, collectively termed the coral holobiont (Margulis, 1991). Although coral reefs have only a relatively small spatial extent, their topography and unique primary production support ~25% of all described marine species (Knowlton and Jackson, 2001). However, coral reefs are also critically threatened habitats currently experiencing significant global declines attributed to localised impacts such as poor water quality including elevated nutrients, sediments and pollutants, and global climate shifts driving elevated sea surface temperatures and ocean acidification ((De'ath *et al.*, 2012) and references

therein). Scleractinian corals live so close to their upper thermal limit that even slight temperature changes can result in large-scale bleaching events (Hoegh-Guldberg *et al.*, 2007; Hughes *et al.*, 2017). Whether or not coral reefs can adapt *sensu lato* to these cumulative environmental pressures is a topic of current debate. The sexual generation time for most reef species (~4–8 years in corals) is generally considered too long for genetic adaptation to keep pace with current rates of climate change (Reusch, 2014). However, recent evidence of increased bleaching tolerance or inheritance of heat tolerance indicates that at least some reef species have the ability to rapidly (days to years) respond to changing climatic conditions ((Palumbi *et al.*, 2014); Supplementary Table 1).

## Adaptation *sensu lato* in the coral holobiont

Broad-sense adaptation to environmental change may occur through both genetic (that is, evolutionary adaptation) and non-genetic (that is, acclimatisation) mechanisms (Box 1). Genetic adaptation is often described as hard inheritance because it involves a

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**Box 1** Defining evolutionary concepts:

Evolutionary adaptation = adaptation *sensu stricto* describes a population-level change in the mean phenotype from one generation to the next due to natural selection and involves a genetic change in the form of differing allele frequencies between generations.

Adaptation *sensu lato* describes any transgenerational process that enhances the fitness of the holobiont phenotype, and is comprised of (i) physiological transgenerational acclimatisation, (ii) host evolution, (iii) heritable microbial community changes and (iv) microbial evolution (the latter two components comprise the basis of microbiome-mediated transgenerational acclimatisation). Transgenerational acclimatisation on the host side often involves epigenetic changes.

Acclimatisation is one component of phenotypic plasticity describing the (often reversible) adjustment of an organism's physiology in response to a changing or novel environment.

Phenotypic plasticity (reversible, developmental or transgenerational) describes the ability of the same genotype to adjust its phenotype under different environmental conditions without genetic change. The reversible physiological component of plasticity corresponds to acclimatisation.

Epigenetics describes environmentally induced changes not encoded in the base sequence of the DNA that may nevertheless alter gene expression levels and have a heritable component. Among the possible mechanisms are DNA-methylation marks, histone acetylation and microRNAs.

Reaction norms describe the particular value of an organismal trait as a function of the environment, with the precise shape determining the plasticity of that trait.

The Price equation is an algorithm describing how the distribution of any characteristic (for example, genes, traits) changes in a biological population from one generation to the next. The basis of the Price equation:  $\Delta z = 1/w \text{cov}(w_i, z_i) + 1/w E(w_i \Delta z_i)$  is that a change in trait  $z$  ( $\Delta z$ ) is determined by the covariance between each value of  $z$  ( $z_i$ ) and fitness ( $w_i$ ), and the expected change in the trait value due to fitness ( $w_i$ ), ( $E(w_i \Delta z_i)$ ).  $w$  represents mean fitness,  $E$  represents the population mean and  $\text{cov}$  represent the population covariance. The simple Price equation approach has been modified to a Price reaction norm approach (Govaert *et al.*, 2016), and accommodates extinction and novel species within the community (Fox and Kerr, 2012).

Tolerance or phenotypic buffering describes how a genotype may function equally under 'good' and 'adverse' environmental conditions.

change in the genotype pool from one generation to the next through natural selection, a process that occurs at the population level. The mechanistic basis is a change in the DNA or RNA sequence, which then changes gene function and/or gene expression, ultimately resulting in altered mean phenotypes. By definition, the adapted population then displays increased Darwinian fitness. In host organisms such as long-lived corals, this is supposed to be a slow process, notwithstanding the potential for within-colony (epi)genetic changes (van Oppen *et al.*, 2015). Somatic mutations as one possible route to 'within-coral colony' adaptation has also recently been proposed and merits further investigation to assess its ubiquity in coral reef systems (Schweinsberg *et al.*, 2015).

Phenotypic plasticity is the ability of the same genotype to adjust its phenotype under different environmental conditions without genetic change, with the reversible component of phenotypic plasticity corresponding to acclimatisation. 'Classical' within-

generation acclimatisation involves an environmentally induced phenotypic response that alters individual fitness with no genetic change at the DNA level. An example would be acclimatisation within the same coral colony to different light levels (Ulstrup *et al.*, 2006). There is increasing evidence however, that acclimatisation can also be transgenerational, for example, by altering chromatin structure or DNA methylation, which can also change gene expression (epigenetic changes, see Putnam *et al.*, 2016). Acclimatisation can act during different ontogenetic stages, which distinguishes it from developmental plasticity that triggers non-reversible ontogenetic changes in particular phases of the life cycle (Gilbert *et al.*, 2015).

Here, we stress that long-term acclimatisation of reef organisms may also be enabled via changes in the associated microbial species, which can evolve much more rapidly than the host, and which can also alter holobiont phenotype by frequency shifts and switching. Rapidly dividing symbiotic microbes (some bacteria divide every 20 min) are predicted to undergo adaptive evolution within weeks to months (Elena and Lenski, 2003), which is two to three orders of magnitude faster than genetic adaptation at the population level for the coral hosts. We call this mechanism microbiome-mediated acclimatisation, which if vertically transmitted would enable microbiome-mediated transgenerational acclimatisation (MMTA) of reef species. Microbially induced modifications of the host phenotype that alter host fitness and can be under selection pressure, has previously been proposed as part of the hologenome theory of evolution (Rosenberg and Zilber-Rosenberg, 2013). Here, we are particularly focussed on microbial contributions to transgenerational acclimatisation of reef species.

*Functional importance of reef microbiomes*

Recent molecular advances have revealed just how fundamentally important microorganisms are to the holobiont phenotype of almost all reef species. For instance, through translocation of photosynthetic assimilates and essential nutrients, the relationship between corals and their symbiotic dinoflagellates shape the health, tolerance limits and phenotypic plasticity of the coral holobiont. Similarly, the functional basis for most reef bacterial symbioses centres on cycling of essential nutrients such as carbon, nitrogen, sulphur and phosphate in addition to passage of trace metals, vitamin synthesis, provision of other cofactors and production of secondary metabolites (reviewed in (Bourne *et al.*, 2016; Webster and Thomas, 2016)). At the other end of the interaction spectrum, colonisation by opportunistic and pathogenic microbes can result in diseased host phenotypes (Bourne *et al.*, 2016).

Yet, despite the large body of literature describing the functional importance of microorganisms to the health and survival of reef species, to date, almost no research has explored the contribution of prokaryotic microorganisms to host transgenerational

### Box 2 Insights derived from other model systems:

The intensely studied human microbiome provides several analogous examples of the microbiome-mediated transgenerational acclimatisation concept. For example, a shift towards a diet high in simple carbohydrates in humans selected for a microbiome that could breakdown and absorb these compounds in the small intestine. Humans who outcompeted the microbes for these substrates had higher fitness, and this interaction between host genetics, environment (in this case diet) and the microbiome has ultimately shaped global human health (Walter and Ley, 2011). Similarly, there are numerous examples of how the human microbiome has adapted to our ecology. For example, a clear demarcation exists in the composition of gut *Bacteroidetes* between African and Western children, reflecting their contrasting diets. Local adaptation of the host can also occur via symbiont acquisition of novel genes, beautifully illustrated in the example of the porphyran-degrading gene acquired in *Bacteroides plebeius* from Japanese populations, enabling them to digest edible seaweed (Hehemann *et al.*, 2010). It is anticipated that the insights derived from one of the most intensively studied model systems—humans—are just the tip of the iceberg with respect to discoveries of microbial contributions to host adaptation in complex microbiomes such as those occurring in reef holobionts.

acclimatisation under changing environmental conditions. Although not a reef species, recent research on the microbiome of the model cnidarian *Nematostella vectensis* certainly suggests the microbiome plays a key role in host acclimatisation and maintenance of holobiont homeostasis under different environmental conditions (Mortzfeld *et al.*, 2016). We know that future climate change conditions can rapidly alter the composition and function of reef host microbiomes (Supplementary Table 1 and reviewed in (Bourne *et al.*, 2016; Webster and Thomas, 2016)), but whether these shifts in symbiosis are causally responsible for the ability of the host to acclimatise or adapt to new environmental conditions is still uncertain. Most researchers have focussed on a loss of symbionts correlating with declining host health. However, favourable symbiotic shifts that enhance the scope for growth or infer a competitive advantage to the host could also be passed to subsequent generations, ultimately enabling MMTA. Importantly, in addition to improved host fitness (Box 2), microbial symbionts may also confer environmental stress tolerance to their hosts (Dunbar *et al.*, 2007), thus effectively modifying the ecological niche of the holobiont (Kopac and Klassen, 2016). The ‘coral probiotic hypothesis’ also posits that alterations in the coral microbiome may enable the host to avoid pathogenic infection under elevated seawater temperature (Reshef *et al.*, 2006).

### Acclimatisation via symbiont shuffling

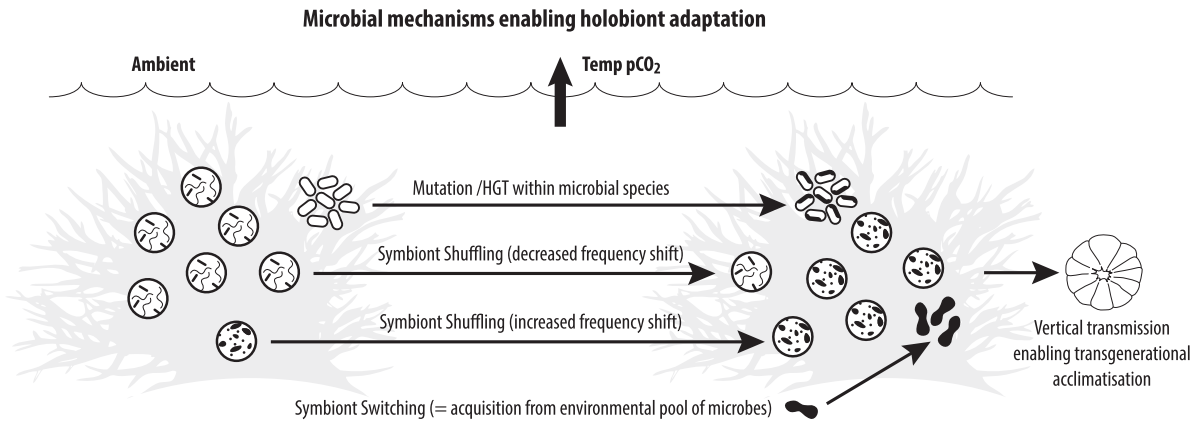
Variation in microbial symbiosis that contributes to local acclimatisation of the host can occur via microbial frequency shifts (symbiont shuffling), acquisition of novel microbial strains (symbiont switching) or horizontal gene transfer (HGT) between

species (Figure 1). These processes can occur very rapidly when environmental conditions change, which may be an important element in the evolution of reef species. For instance, environmentally mediated microbial amplification was recently reported from a natural shallow water CO<sub>2</sub> seep in Papua New Guinea (Morrow *et al.*, 2015). In this environment, two species of reef sponge were found to be 40-fold more abundant at sites with active CO<sub>2</sub> bubbling than they were at control sites only 100’s of metres away. Importantly, increased host abundance at the seep correlated with a significantly higher relative abundance of photosynthetic *Synechococcus* within the sponge holobionts at this site. This frequency shift of photosynthetic microorganisms at the seep likely provided the sponges with a greater nutritional benefit under future conditions of ocean acidification. Similarly, corals are known to harbour a diverse suite of symbiotic *Symbiodinium* that have distinct physiological optima, and shuffling the relative abundance of these symbionts within the holobiont provides corals with a mechanism to adjust their thermal tolerance (Baird *et al.*, 2007). This community shift is evidenced by numerous studies documenting increased relative abundance of heat-tolerant *Symbiodinium* type D1 (LaJeunesse *et al.*, 2014) in corals exposed to elevated seawater temperatures (Supplementary Table 1; (Howells *et al.*, 2016); and references therein).

### Acclimatisation via symbiont switching

Scleractinian corals are constantly exposed to a diverse suite of microorganisms present in their surrounding environment and, in some instances, these microbes may establish a new host niche enabling them to impact holobiont phenotype under altered environmental conditions. A compelling example is the selection of optimal coral algal symbionts from the surrounding environment depending on prevailing environmental conditions (Boulotte *et al.*, 2016). Coral symbiont switching to a thermally resistant *Symbiodinium* clade was potentially driven by multiple preceding bleaching events (Boulotte *et al.*, 2016). Switching of bacterial symbionts has also been observed in marine sponges and Foraminifera. For instance, when exposed to high-nutrient and low-salinity conditions over 12 months, the reef sponge *Rhopaloeides odorabile* replaces many of its native symbionts including the *Poribacteria*, with microorganisms having high similarity to symbionts of sponges known to inhabit more nutrient-rich environments (Webster *et al.*, 2011). Similarly, the Foraminifera *Heterostegina depressa* switches between different *Acidomicrobiales* symbionts at different temperature and pCO<sub>2</sub> levels (Webster *et al.*, 2016). While MMTA still needs to be assessed for these examples, it is notable that both the sponges and Foraminifera are able to maintain their health for extended durations following





**Figure 1** Conceptual overview of the microbial mechanisms facilitating transgenerational acclimatisation of coral reef organisms. Under elevated seawater temperature and  $p\text{CO}_2$ , specific host-associated microorganisms may increase or decrease in abundance, thereby altering holobiont function and fitness. These frequency shifts can occur in the *Symbiodinium* (large circular cells differentially shaded according to clade) or prokaryotic community (rod-shaped cells). Shifts in community composition can also occur via acquisition of new microorganisms from the surrounding environment. In addition, mutation and/or horizontal gene transfer can introduce new genetic material into existing symbiont populations (white cells with black inset). This process can occur in any component (*Symbiodinium*, bacteria, archaea) of the host microbiome. If microbial alterations that infer a fitness advantage to the host are vertically transmitted to the offspring (denoted by sketch of a newly settled coral recruit), transgenerational acclimatisation can occur.

symbiont switching (Webster *et al.*, 2011), and the sponge species is also known to vertically transmit the majority of its complex microbiome (Webster *et al.*, 2010). Importantly, symbiont shuffling and symbiont switching are not mutually exclusive; rather it is likely that both mechanisms operate in tandem. While the ability of reef organisms to associate with functionally diverse symbionts contributes to their success across a range of environmental conditions, assessments of heritable variation in tolerance are still required to estimate future evolutionary potential (Reusch, 2014).

### Adaptation via genetic mutation within symbiont lineages

Because of their rapid division rates, symbiotic microbes are much more prone to adaptive evolution within their mitotically dividing and sexual phases than their eukaryotic hosts. Sexual recombination in *Symbiodinium* has recently been suggested based on the analysis of DNA polymorphisms (Wilkinson *et al.*, 2015) and genome analysis revealing numerous meiosis-specific genes (Chi *et al.*, 2014). However, direct observational evidence or single-cell genotyping approaches that target multiple, single-copy loci are still required for conclusive evidence for inter-lineage recombination and introgression. Regardless of the mechanism, *Symbiodinium* populations have been shown to influence holobiont tolerance through physiological and genetic adaptation to prevailing conditions (Supplementary Table 1; (Levin *et al.*, 2016); and references therein). Several physiological and transcriptional differences (related to photosynthetic efficiency, reactive oxygen species and molecular chaperones) exist between thermo-sensitive C1 and thermo-tolerant C1 *Symbiodinium* populations that possibly have a genetic basis. Both populations display

an enrichment of meiosis functional gene groups at elevated temperature, which is important in promoting adaptation (Levin *et al.*, 2016) compared to purely asexually dividing strains (Kaltz and Bell, 2002). Adaptive variation in thermal tolerance of asexual *Symbiodinium* C1 cultures is also retained following a year of growth at lower temperatures (Howells *et al.*, 2012). A novel experimental evolution study recently confirmed the possibility of adaptive evolution enhancing thermal tolerance in *Symbiodinium* symbionts ex-hospite (Chakravarti *et al.*, 2017). Significant adaptive potential therefore exists if the rates of somatic mutation in *Symbiodinium* are found to be similar to other species of single-celled eukaryotic algae (Costas *et al.*, 2008), especially considering their fast asexual generation times and large population sizes within corals. Furthermore, elevated seawater temperatures associated with global climate change likely provide strong selective pressure favouring the persistence and proliferation of thermally tolerant genetic variants. Nothing is known thus far on ‘within-host’ evolution of associated bacteria, yet they may evolve even faster than eukaryotic microbes because of ample mechanisms of gene acquisition via HGT (see below) and since any mutation is immediately visible to selection in haploid bacteria and archaea.

### Adaptation via HGT

The mutational basis of genomic change within microbial populations may be greatly enhanced by mechanisms of HGT. Conjugation and transformation provide mechanisms for modification of bacterial DNA with inter-species ‘mating’ and natural competence being two major mechanisms driving HGT in bacteria. Most microorganisms also possess different classes of mobile genetic elements that allow for the acquisition, loss or rearrangement of

regions of the bacterial genome. For instance, recent analysis of the human microbiome revealed pervasive mobile genes with functional variation across human populations corresponding to known dietary differences (Chi *et al.*, 2014), highlighting environment as a strong selective pressure in shaping mobile gene pools. In addition, viruses can shuttle these genes between different components of the holobiont via a third HGT mechanism, transduction (Hambly and Suttle, 2005). Viral-mediated HGT of beneficial genes (between different symbiotic microorganisms and/or between host and symbionts) has the potential to increase host fitness and cause rapid ecological speciation that allows reef holobionts to adapt and survive under changing environmental conditions. While HGT among prokaryotes and viruses is ubiquitous, cases of HGT among eukarya/metazoan hosts and prokaryotes are less clear. One excellent example of suspected HGT in sponges involves a spherulin-encoding gene being transferred from a microorganism to the host (Jackson *et al.*, 2011). Spheruline is thought to be involved in skeletogenesis, indicating that this HGT event may have contributed to the evolution of the sponge body plan. Another example, is the suspected transfer of eukaryotic-like proteins from the sponge host to the symbionts, with these eukaryotic-like proteins thought to be used by the symbionts to regulate host phagocytosis (Nguyen *et al.*, 2014). These examples demonstrate the potential for HGT in reef holobionts, and future examination of existing and emerging reef genomic data (such as that being generated by the ReFuGe 2020 consortium; (Voolstra *et al.*, 2015)) is expected to reveal more specific examples related to environmental adaptation.

## A conceptual framework for predicting holobiont acclimatisation and adaptation

To assess the relative importance of the mechanisms leading to adaptation in reef organisms via MMTA, we recommend employing the Price equation (Price, 1970). The Price equation has been applied in community ecology to disentangle the relative importance of within-species changes owing to plasticity based on genetic adaptation and community change, for the functioning (for example, biomass production) of that community (Collins and Gardner, 2009; Govaert *et al.*, 2016). This is achieved by viewing the holobiont's microbial community as plastic, describing how the distribution (for example, mean, variance, and so on) of any characteristic (for example, genes, trait values, and so on) changes in a biological population between generations. The Price equation partitions phenotypic responses into (i) physiological, (ii) ecological and (iii) evolutionary components, and this quantitative framework therefore provides a conceptual tool for understanding and predicting the acclimatisation and adaptation of the holobiont to global

change. For instance, the composition/function (i—physiological) and abundance (ii—ecological) of a coral-associated microbiome may undergo rapid shifts as environmental conditions change, and microbial lineages that survive exposure to elevated seawater temperature and/or ocean acidification may also undergo microevolution (iii—evolutionary), all of which ultimately impacts the holobiont phenotype under climate change. Note that the compositional term integrates two processes, the acquisition and local extinction of symbionts and shifts in relative frequency of the original community (Fox and Kerr, 2012).

The Price equation approach has recently been successfully combined with reaction norm analysis (relating mean phenotypic response of a genotype to the change in environment) (Govaert *et al.*, 2016). This combined Price equation/reaction norm metric should successfully disentangle non-evolutionary and evolutionary contributors, and their interaction to trait change (phenotypic plasticity, species sorting and the interaction between species sorting and evolutionary components) within reef holobionts. Note that currently, the Price equation approach is only strictly valid for traits as state variables, for example, contributions to the energy allocation of a coral polyp. Depending on the response variable upon environmental disturbance, modifications of the partitioning approach would need to be developed. For instance, if rates (for example, photosynthesis rates) were of interest, current approaches would need to be modified to represent such variables. Although it may be difficult in complex natural systems to measure all required components of within- and among microbial symbiont change, it is nevertheless conceptually useful and may guide simplified experiments to integrate MMTA across levels of biological organisation. We also suggest broadening the Price equation/reaction norm concept by allowing for genetic exchange within the microbiome via HGT.

## Hologenome concept

Adaptation *sensu lato* as discussed here encompasses any fitness gain of the collective phenotype under novel stressful conditions. In line with the emerging consensus on the hologenome theory and levels of selection (Bordenstein and Theis, 2015; Moran and Sloan, 2015; Kopac and Klassen, 2016; Douglas and Werren, 2016; Theis *et al.*, 2016), this does not require co-evolution of the entire holobiont as an individual unit. Rather, specific microorganisms or microbial genes that provide a fitness advantage to the host under conditions of elevated sea temperature and ocean acidification could be maintained and passed to subsequent generations, ultimately resulting in transgenerational acclimatisation of the host. For instance, it has been established that corals can effectively regulate their internal pH enabling them to at least partially mitigate the effects of ocean

acidification (McCulloch *et al.*, 2012). However, the physiological or energetic cost of this response may only be sustainable in the longer term if there is a shift in the symbiotic microbiome towards a community that meets the increased energetic demand. The holobiont would not only be better acclimatised to its local environment, but would also have an increased probability of passing on the acquired microbial traits to the next generation. Thus, the individual evolutionary trajectories of the different holobiont members can still enable transgenerational acclimatisation of a reef organism, regardless of whether the holobiont is heritable as a unit. Reef holobionts can assemble their diverse microbiomes to maximise host fitness, and then environmental forces can select for reassembly or new membership as required.

### Symbiont transmission facilitating transgenerational acclimatisation

For microbial species to play a role in MMTA, they must be faithfully transmitted between generations (vertical transmission) or dependably acquired from the environment in each new generation (horizontal transmission). A major consideration in the hologenome debate is that higher levels of selection are most likely to apply to particular host-symbiont pairs with strict partner fidelity rather than applying to the entire microbiome. Variation in partner fidelity undoubtedly occurs across the complex microbiomes of reef species such as corals and sponges, and it is highly unlikely that the entire microbiome of a reef organism would evolve as a holobiont unit with mutually beneficial coadaptation. However, in a large number of reef species there is support for high partner fidelity in at least some components of the complex microbiome, with particular microorganisms inhabiting corals, sponges and ascidians being vertically transmitted (Supplementary Table 1 and reviewed in (Bourne *et al.*, 2016; Webster and Thomas, 2016)). Some reef species also reproduce asexually via fragmentation or production of asexual gemmules, so that each of the fragments inherits the same microbiome as the adult population. However, MMTA is unlikely to be ubiquitous across all reef species as it can only occur if there is strict vertical transmission of the altered microbiome, and some coral species actually obtain new symbionts from the surrounding environment each generation. For instance, recent analysis of coral and algal microbiomes revealed communities of highly diverse and stable symbionts, but no single bacterial species that was ubiquitously associated with a single host species, indicating a lack of strict symbiont heredity (Hester *et al.*, 2016). Unfortunately, however, in most reef species, there is still little understanding of how stringent the vertical transmission process is, and whether these populations persist or change during larval metamorphosis and subsequent juvenile development.

### Functional equivalence and evolutionary convergence in reef microbiomes

Until recently, it was also unknown whether the diverse microbiomes of reef species shared functions, and if so, whether these equivalent functions were undertaken by evolutionary convergent mechanisms (Webster and Thomas, 2016). However, recent research on sponge microbiomes found that despite the species-specific taxonomic profiles that are characteristic of sponges (Thomas *et al.*, 2016), core functions such as nitrogen metabolism and nutrient utilisation were shared across phylogenetically disparate microorganisms (Fan *et al.*, 2012; Ribes *et al.*, 2012). Microbial communities in divergent hosts seemingly evolved different genomic solutions to undertake the same function or inhabit the same niche with many of these core symbiont functions being provided by equivalent enzymes and biosynthetic pathways (Fan *et al.*, 2012). Even when free-living microorganisms are initially able to enter into low selectivity or random (that is, neutral) partnerships with the host, as the symbiotic relationship evolves and vertical transmission occurs, symbionts should maintain or acquire functions that stabilise their interaction with their host. Thus, for different host species with similar functional niches, symbionts will eventually converge functionally and the collective gene pool encompassed within the holobiont is therefore critical for transgenerational acclimatisation of the host.

### Conclusion

We propose here an inclusive concept of adaptation *sensu lato* in coral reef organisms, that encompasses in addition to (epi)genetic adaptation of the host, a pervasive role of MMTA via symbiont shuffling and switching, genetic mutation, and horizontal transfer of beneficial genes that are inherited by offspring. Successful modelling of future reefs therefore requires an accurate assessment of several as yet understudied holobiont processes (Figure 1). To date, most experimental research has focussed on determining the phenotypic responses of reef organisms in single-factor, short-term, single generation experiments, severely limiting our ability to assess the potential for evolutionary adaptation. Recommendations for future research on reef species therefore include (i) determining if variation in host fitness due to assembly of different microbiomes with unique microbial functions ultimately drives a multigenerational response to selection, (ii) experimentally assessing the mechanisms and rates of transgenerational acclimatisation and adaptation, (iii) manipulative experiments to alter microbial composition and assess phenotypic responses, (iv) determining the extent of functional equivalence in microbiomes to assess whether hidden diversity is critical for holobiont functioning under ocean change, and (v) using a modified Price equation (Price, 1970; Govaert *et al.*,



2016) to integrate the relative contributions of (epi)genetic adaptations in the host via (a) germ line and mitotic mutations, (b) microbiome changes established by both shuffling and switching and (c) evolution within microbial strains including HGT.

## Conflict of Interest

The authors declare no conflict of interest.

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## References

- Baird AH, Cumbo VR, Leggat W, Rodriguez-Lanetty M. (2007). Fidelity and flexibility in coral symbioses. *Mar Ecol Prog Ser* **347**: 307–309.
- Bordenstein SR, Theis KR. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol* **13**: e1002226.
- Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM *et al.* (2016). Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. *ISME J* **10**: 2693–2701.
- Bourne DG, Morrow KM, Webster NS. (2016). Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annu Rev Microbiol* **70**: 317–340.
- Chakravarti LJ, Beltran VH, vanOppen MJH. (2017). Rapid thermal adaptation in photosymbionts of reef-building corals. *Glob Change Biol*; e-pub a head of print 27 April 2017; doi:10.1111/gcb.13702.
- Chi J, Parrow MW, Dunthorn M. (2014). Cryptic sex in *Symbiodinium* (Alveolata, Dinoflagellata) is supported by an inventory of meiotic genes. *J Eukaryot Microbiol* **61**: 322–327.
- Collins S, Gardner A. (2009). Integrating physiological, ecological and evolutionary change: a Price equation approach. *Ecol Lett* **12**: 744–757.
- Costas E, Flores-Moya A, López-Rodas V. (2008). Rapid adaptation of phytoplankters to geothermal waters is achieved by single mutations: were extreme environments 'Noah's Arks' for photosynthesizers during the Neoproterozoic 'snowball Earth'? *New Phytol* **180**: 922–932.
- De'ath G, Fabricius KE, Sweatman H, Puotinen M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc Natl Acad Sci USA* **109**: 17995–17999.
- Douglas AE, Werren JH. (2016). Holes in the hologenome: why host-microbe symbioses are not holobionts. *MBio* **7**: e02099–15.
- Dunbar HE, Wilson ACC, Ferguson NR, Moran NA. (2007). Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biol* **5**: e96.
- Elena SF, Lenski RE. (2003). Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nat Rev Genet* **4**: 457–469.
- Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS *et al.* (2012). Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. *Proc Natl Acad Sci USA* **109**: E1878–E1887.
- Fox JW, Kerr B. (2012). Analyzing the effects of species gain and loss on ecosystem function using the extended Price equation partition. *Oikos* **121**: 290–298.
- Gilbert SF, Bosch TCG, Ledon-Rettig C. (2015). Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. *Nat Rev Genet* **16**: 611–622.
- Govaert L, Pantel JH, De Meester L. (2016). Eco-evolutionary partitioning metrics: assessing the importance of ecological and evolutionary contributions to population and community change. *Ecol Lett* **19**: 839–853.
- Hambly E, Suttle CA. (2005). The virosphere, diversity, and genetic exchange within phage communities. *Curr Opin Microbiol* **8**: 444–450.
- Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czekaj M, Michel G. (2010). Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* **464**: 908–912.
- Hester ER, Barott KL, Nulton J, Vermeij MJA, Rohwer FL. (2016). Stable and sporadic symbiotic communities of coral and algal holobionts. *ISME J* **10**: 1157–1169.
- Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E *et al.* (2007). Coral reefs under rapid climate change and ocean acidification. *Science* **318**: 1737–1742.
- Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH. (2012). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nat Clim Chang* **2**: 116–120.
- Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA. (2016). Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Glob Chang Biol* **22**: 2702–2714.
- Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH *et al.* (2017). Global warming and recurrent mass bleaching of corals. *Nature* **543**: 373–377.
- Jackson DJ, Macis L, Reitner J, Worheide G. (2011). A horizontal gene transfer supported the evolution of an early metazoan biomineralization strategy. *BMC Evol Biol* **11**: 238.
- Kaltz O, Bell G. (2002). The ecology and genetics of fitness in *Chlamydomonas*. XII: repeated sexual episodes increase rates of adaptation to novel environments. *Evolution* **56**: 1743–1753.
- Knowlton N, Jackson JBC. (2001). The ecology of coral reefs. In: Bertness MD, Gaines SD, Hay ME (eds). *Marine Community Ecology*. Sinauer Associates Incorporated: Sunderland, MA, USA, pp 395–422.
- Kopac SM, Klassen JL. (2016). Can they make it on their own? Hosts, microbes, and the holobiont niche. *Front Microbiol* **7**: 1647.
- LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA. (2014). Ecologically

- differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) clade D are different species. *Phycologia* **53**: 305–319.
- Levin RA, Beltran VH, Hill R, Kjelleberg S, McDougald D, Steinberg PD *et al.* (2016). Sex, scavengers, and chaperones: transcriptome secrets of divergent *Symbiodinium* thermal tolerances. *Mol Biol Evol* **33**: 2201–2215.
- Margulis L. (1991). Symbiogenesis and symbiogenesis. In: Margulis L, Fester R (eds). *Symbiosis AS A Source of Evolutionary Innovation: Speciation and Morphogenesis*. MIT Press: Cambridge, MA, USA, pp 1–14.
- McCulloch M, Falter J, Trotter J, Montagna P. (2012). Coral resilience to ocean acidification and global warming through pH up-regulation. *Nat Clim Chang* **2**: 623–627.
- Moran NA, Sloan DB. (2015). The hologenome concept: helpful or hollow? *PLoS Biol* **13**: e1002311.
- Morrow KM, Bourne DG, Humphrey C, Botté ES, Laffy P, Zaneveld JR *et al.* (2015). Natural volcanic CO<sub>2</sub> seeps reveal future trajectories for host-microbial associations in corals and sponges. *ISME J* **9**: 894–908.
- Mortzfeld BM, Urbanski Sn, Reitzel AM, Künzel S, Technau U, Fraune S. (2016). Response of bacterial colonization in *Nematostella vectensis* to development, environment and biogeography. *Environ Microbiol* **18**: 1764–1781.
- Nguyen MT, Liu M, Thomas T. (2014). Ankyrin-repeat proteins from sponge symbionts modulate amoebal phagocytosis. *Mol Ecol* **23**: 1635–1645.
- Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA. (2014). Mechanisms of reef coral resistance to future climate change. *Science* **344**: 895–898.
- Price GR. (1970). Selection and covariance. *Nature* **227**: 520–521.
- Putnam HM, Davidson JM, Gates RD. (2016). Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evol Appl* **9**: 1165–1178.
- Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E. (2006). The coral probiotic hypothesis. *Environ Microbiol* **8**: 2068–2073.
- Reusch TBH. (2014). Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. *Evol Appl* **7**: 104–122.
- Ribes M, Jiménez E, Yahel G, López-Sendino P, Díez B, Massana R *et al.* (2012). Functional convergence of microbes associated with temperate marine sponges. *Environ Microbiol* **14**: 1224–1239.
- Rosenberg E, Zilber-Rosenberg I. (2013). *The Hologenome Concept: Human, Animal and Plant Microbiota*. Springer: Basel, Switzerland.
- Schweinsberg M, Weiss LC, Striewski S, Tollrian R, Lampert KP. (2015). More than one genotype: how common is intracolony genetic variability in scleractinian corals? *Mol Ecol* **24**: 2673–2685.
- Theis KR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG *et al.* (2016). Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *mSystems* **1**: e00028–16.
- Thomas T, Moitinho-Silva L, Lurgi M, Björk JR, Easson C, Astudillo C *et al.* (2016). Global sponge microbiome: diversity, structure and convergent evolution of symbiont communities across the phylum Porifera. *Nat Commun* **7**: 11870.
- Ulstrup KE, Ralph PJ, Larkum AWD, Kuhl M. (2006). Intra-colonial variability in light acclimation of zooxanthellae in coral tissues of *Pocillopora damicornis*. *Mar Biol* **149**: 1325–1335.
- van Oppen MJH, Oliver JK, Putnam HM, Gates RD. (2015). Building coral reef resilience through assisted evolution. *Proc Natl Acad Sci USA* **112**: 2307–2313.
- Voolstra CR, Miller DJ, Ragan MA, Hoffmann A, Hoegh-Guldberg O, Bourne D *et al.* (2015). The ReFuGe 2020 consortium - using ‘omics’ approaches to explore the adaptability and resilience of coral holobionts to environmental change. *Front Mar Sci* **2**: 1–8.
- Walter J, Ley RE. (2011). The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol* **65**: 411–429.
- Webster NS, Thomas T. (2016). The sponge hologenome. *MBio* **7**: e00135–16.
- Webster NS, Negri AP, Botté ES, Laffy P, Flores F, Noonan S *et al.* (2016). Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. *Sci Rep* **6**: 19324.
- Webster NS, Cobb RE, Soo R, Anthony SL, Battershill CN, Whalan S *et al.* (2011). Bacterial community dynamics in the marine sponge *Rhopaloeides odorabile* under *in situ* and *ex situ* cultivation. *Mar Biotechnol* **13**: 296–304.
- Webster NS, Taylor MW, Benham F, Lückner S, Rattei T, Whalan S *et al.* (2010). Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environ Microbiol* **12**: 2070–2082.
- Wilkinson SP, Fisher PL, van Oppen MJH, Davy SK. (2015). Intra-genomic variation in symbiotic dinoflagellates: recent divergence or recombination between lineages? *BMC Evol Biol* **15**: 1–12.



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